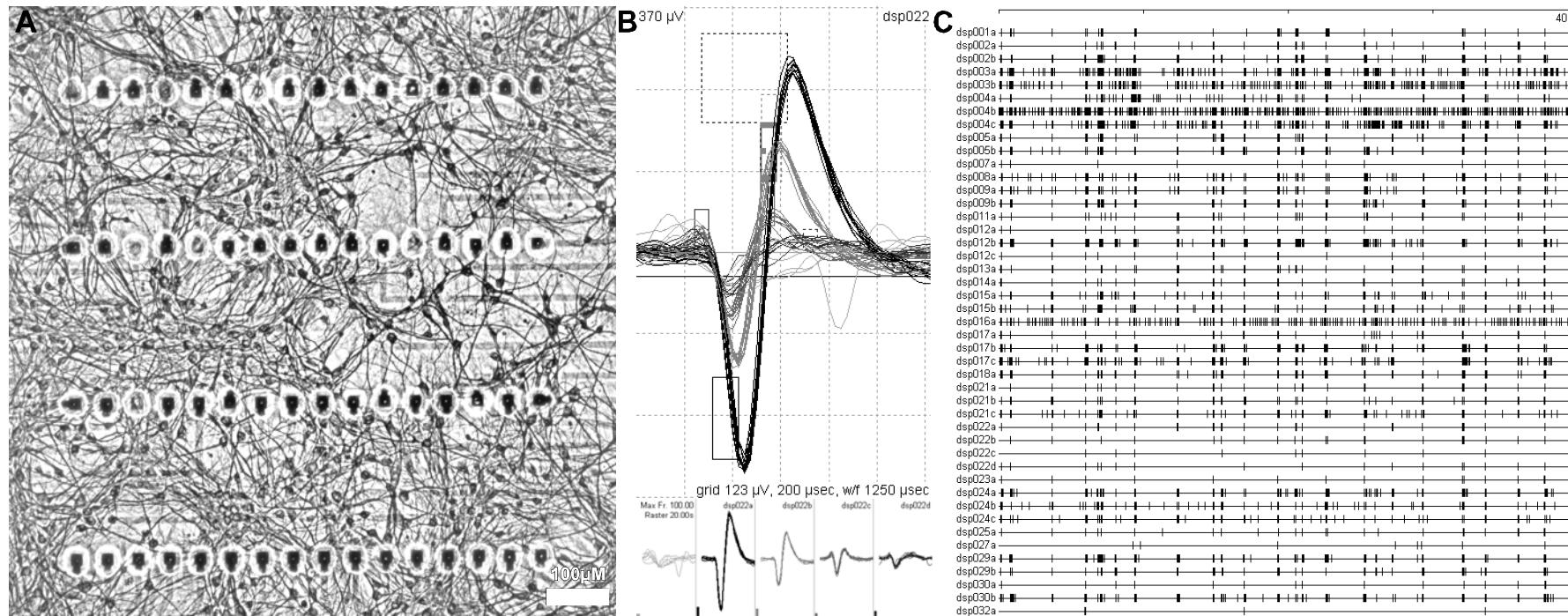
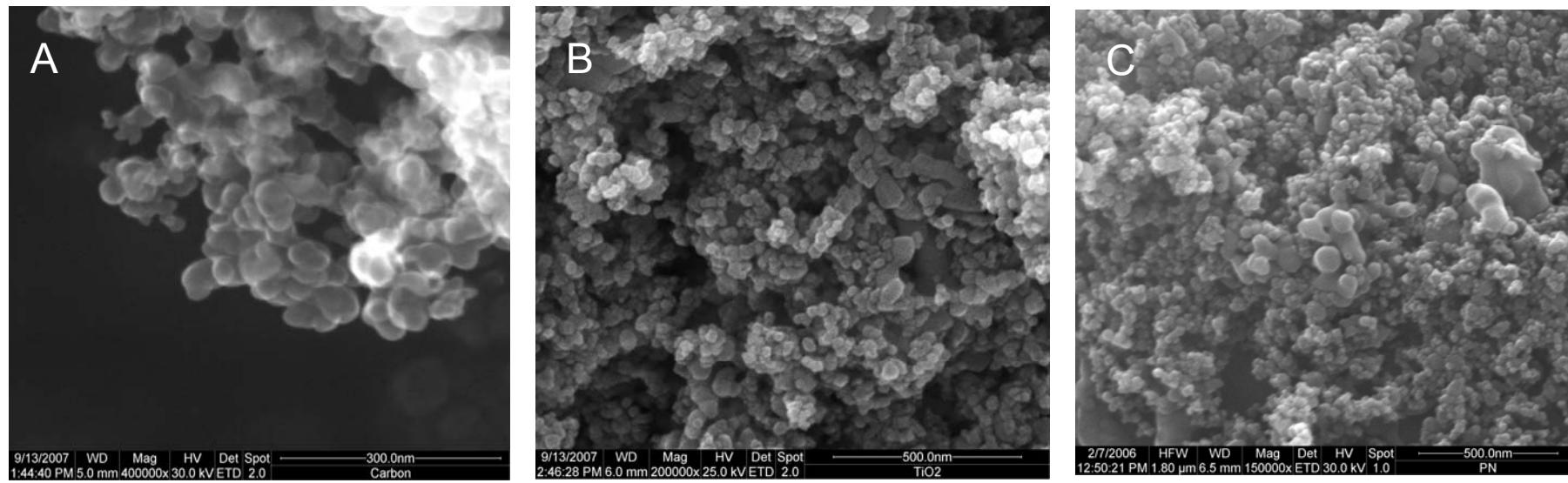


Supplementary Material to Gramowski et al. : Nanoparticles Induce Changes in Neuronal Networks



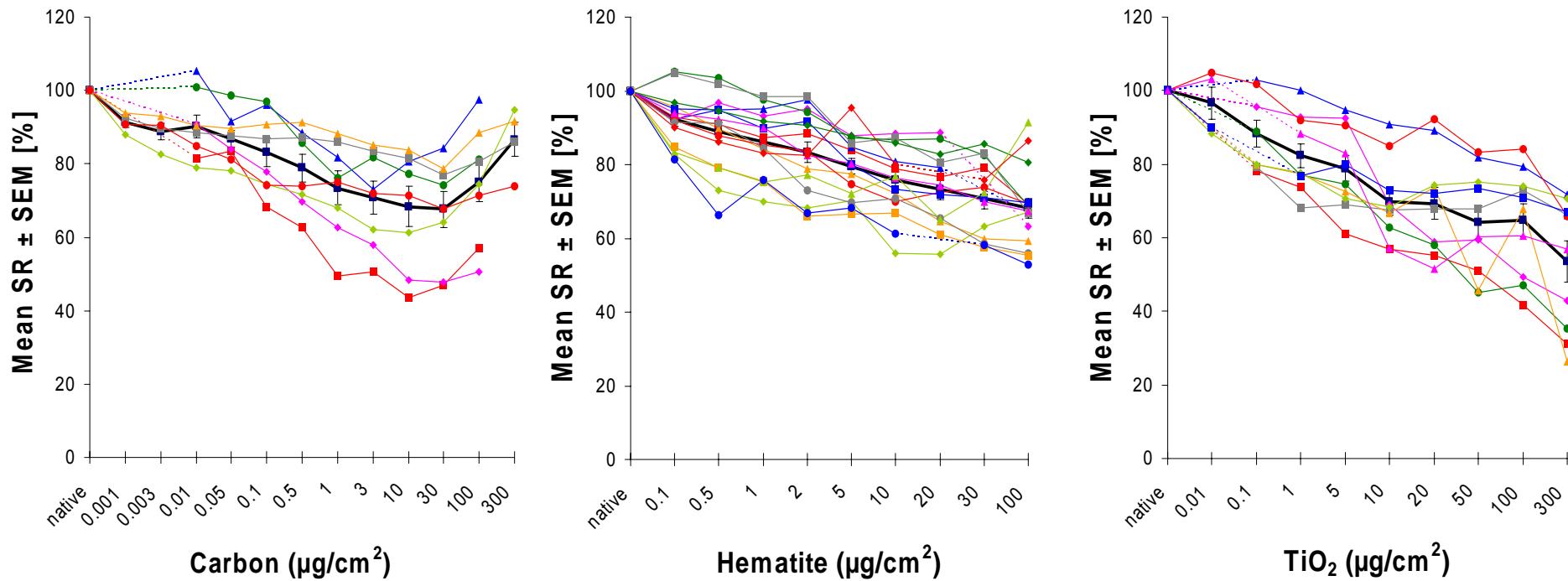
**Figure 1:** (A) Frontal cortex network grown on a 64 electrode MEA 21 days in vitro. The culture was immuno-stained against neurofilament and MAP2 proteins. Electrode diameter: 20  $\mu\text{m}$ , horizontal spacing: 40  $\mu\text{m}$ , vertical spacing: 200  $\mu\text{m}$ . (B) An electrode is able to record action potentials from several neurons (top) which are separated online by the software (bottom). (C) 40 seconds of spontaneous native electrical activity of a frontal cortex culture. Each line represents one neuron where action potentials are indicated by a vertical tick. (Reprinted from Gramowski et al. (2006) with permission from the European Journal of Neuroscience)

Spikes in spike trains are clustered into bursts. These represent the ubiquitous and prominent feature of network activity and are an essential aspect of the neuronal code (Gross 1994; Lisman 1997). A quantitative description of bursts was accomplished through direct spike train analysis with the program NeuroEXplorer (Plexon Inc., Dallas, TX, USA) and the in-house programs Analyzer, Graphs and Squid (NeuroProof GmbH, Rostock, Germany). The maximum spike interval to start a burst (100 ms) and the maximum interval to end a burst (200 ms) were set to define the bursts. The minimum number of spikes was set at 2, and minimum duration was 1 ms.



**Figure 2:** Raster electron microscopy images of the NPs used. **a)** carbon black, **b)** TiO<sub>2</sub>, **c)** hematite (scale bars a, 300 nm, b and c, 500 nm). The average diameter of CB-NP were ≤ 55 nm while both the hematite (Fe<sub>2</sub>O<sub>3</sub>)-NP and TiO<sub>2</sub>-NP had an average diameter of ≤ 100 nm. Following suspension in serum free liquid media for REM the NPs were found to have aggregated to larger sizes.

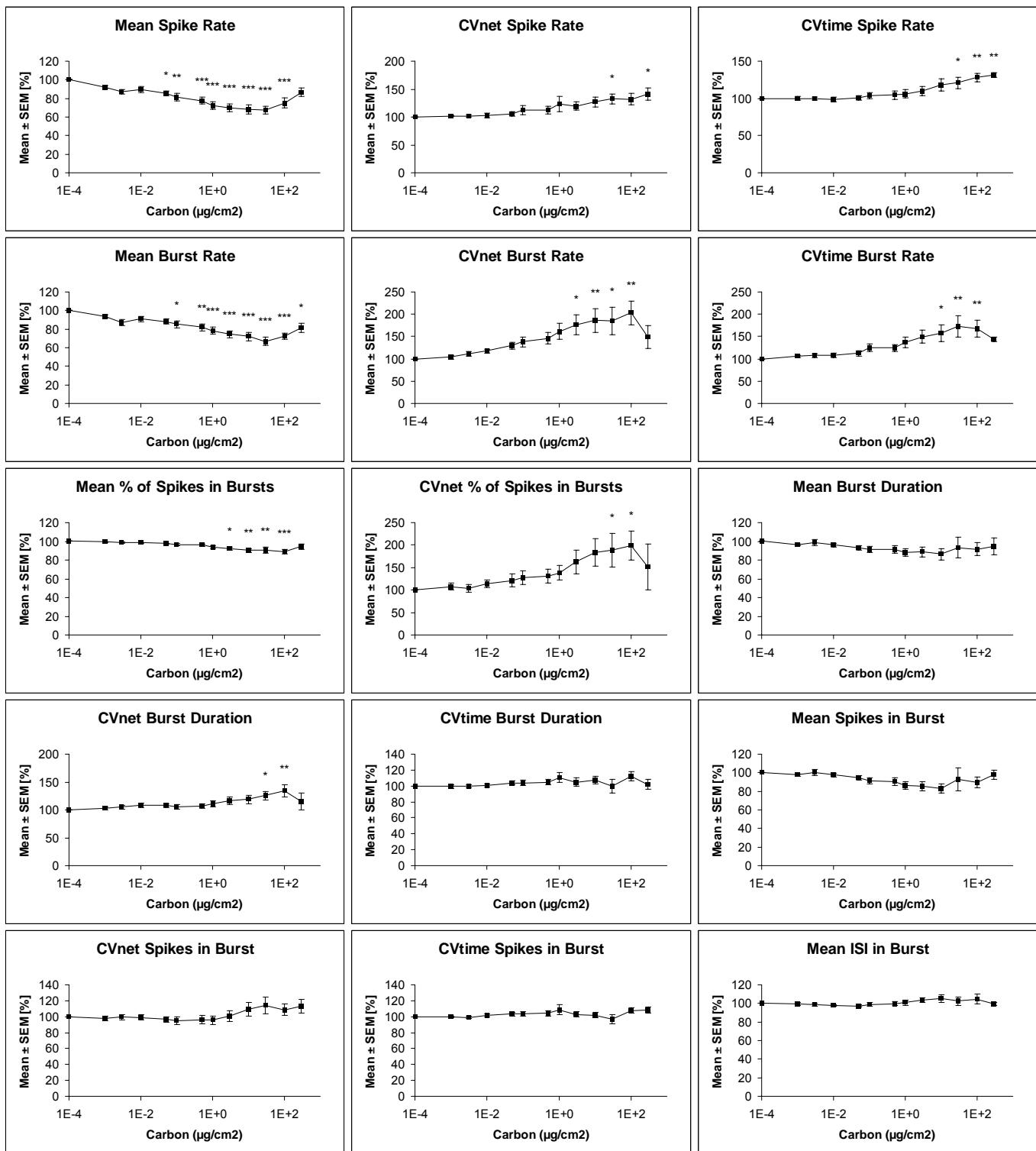
For REM analysis the NP were mounted onto a copper stage and heat treated at <200°C for 30 min to remove moisture from the samples. Elemental analysis of the nanomaterial was performed at the same time with an energy dispersive X-ray microanalysis system (EDX) (KEVEX, Geel, Belgium). Furthermore, intracellularly detected NPs were analyzed by a back scatter detector, which demonstrates the metallic inclusions in light colour against a dark background (BSA mode in REM DSM 960 A).



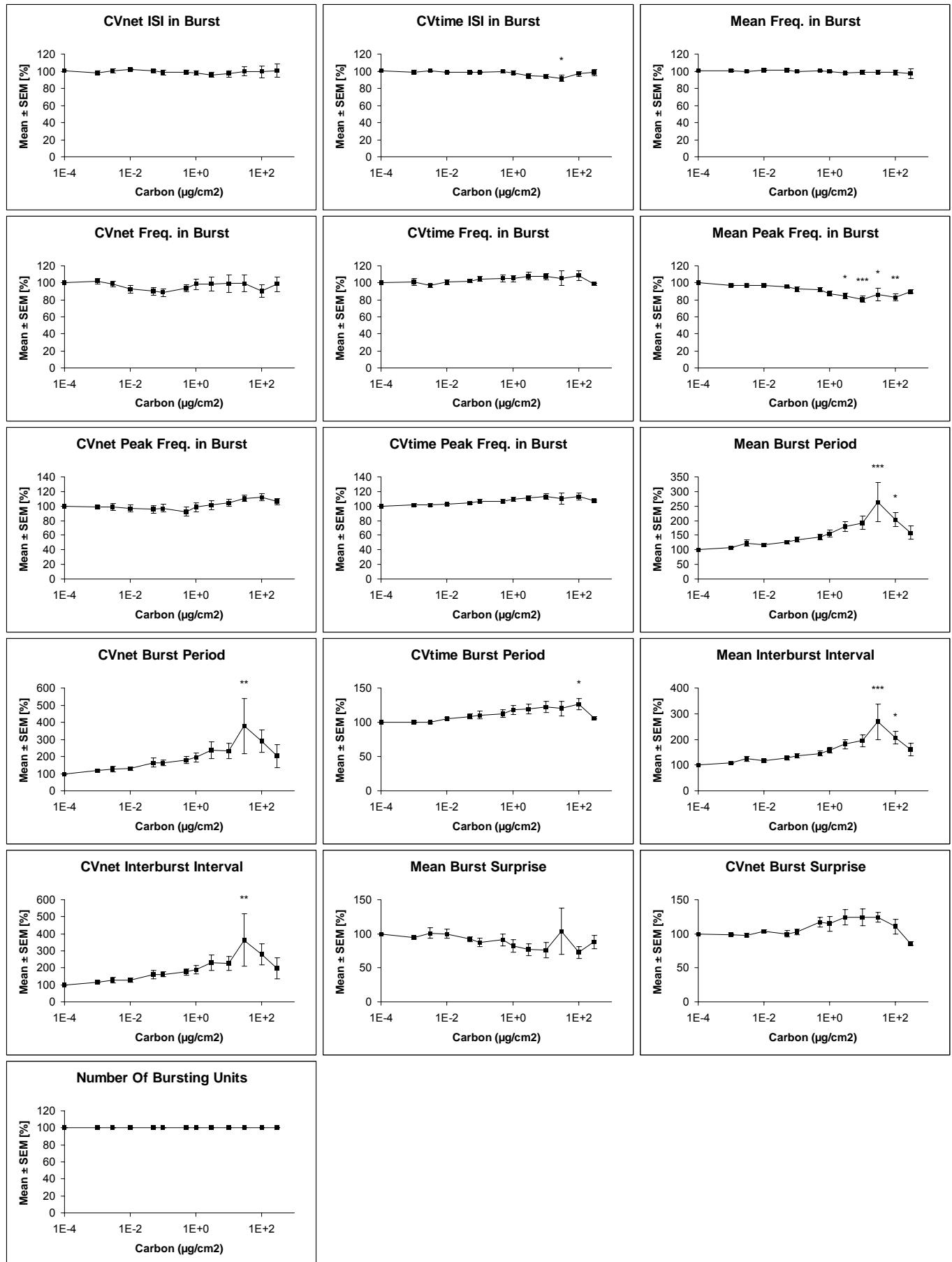
**Figure 3:** Variability of frontal cortex network data (black line: mean value). Data of dose-response curves of spike rate changes caused by CB (n=8 independent experiments); hematite (n=16); and  $\text{TiO}_2$  (n=10). The electrophysiological data were derived from frontal cortex networks originating from 6 separate primary embryonic cell preparations. The parameter values are normalized and given in %s.

**Table 1:** Overview of 31 parameters characterizing the changes of the electrical activity patterns of neuronal networks. Synchronicity and oscillatory behavior were captured through the temporal and network coefficients of variation ( $CV_{TIME}$  and  $CV_{NETWORK}$ ) of the burst rate, burst period, and spike rate parameters. Therefore the variation coefficients quantify the spatiotemporal behavior reflecting temporal dynamics and the fundamental interactions within the networks. Here,  $CV_{TIME}$  reflects the periodic behavior of a single neuron activity pattern.  $CV_{NETWORK}$  describes the coordination between different neurons in a specific activity state and is a measure of synchronicity (Gramowski et al. 2004, Keefer et al. 2001).

General activity	Synchronicity	Oscillatory behaviour
Spike rate	Spike rate $CV_{net}$	Spike rate $CV_{time}$
Burst rate	Burst rate $CV_{net}$	Burst rate $CV_{time}$
% of spikes in burst	% of spikes in burst $CV_{net}$	Burst period $CV_{time}$
Number of bursting units		
Burst structure		
Mean burst duration	Mean burst duration $CV_{net} t$	Mean burst duration $CV_{time}$
Mean number of spikes in burst	Mean number of spikes in burst $CV_{net}$	Mean number of spikes in burst $CV_{time}$
Mean interspike interval in burst	Mean interspike interval $CV_{net}$	Mean interspike interval $CV_{time}$
Mean frequency in burst	Mean frequency in burst $CV_{net}$	Mean frequency in burst $CV_{time}$
Mean peak frequency in burst	Mean peak frequency in burst $CV_{net}$	Mean peak frequency in burst $CV_{time}$
Burst period	Burst period $CV_{net}$	
Interburst interval	Interburst interval $CV_{net}$	
Burst surprise	Burst surprise $CV_{net}$	

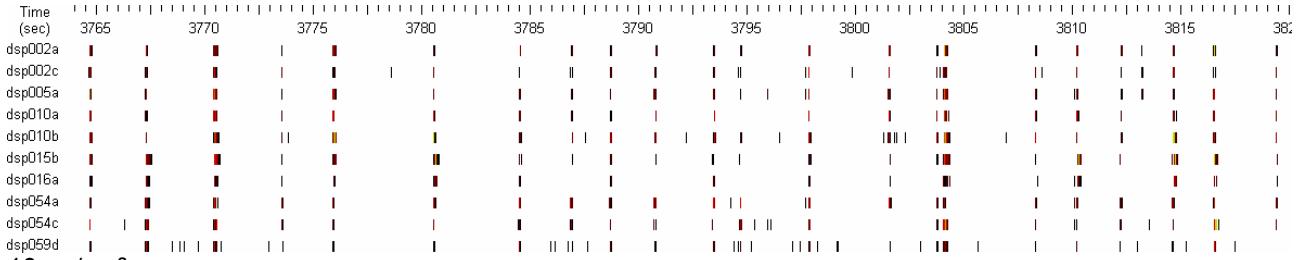


**Figure 4:** Concentration-response curves of 31 activity describing parameters after cumulative application of carbon black nanoparticles. Significance levels after ANOVA Dunnett's test: \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

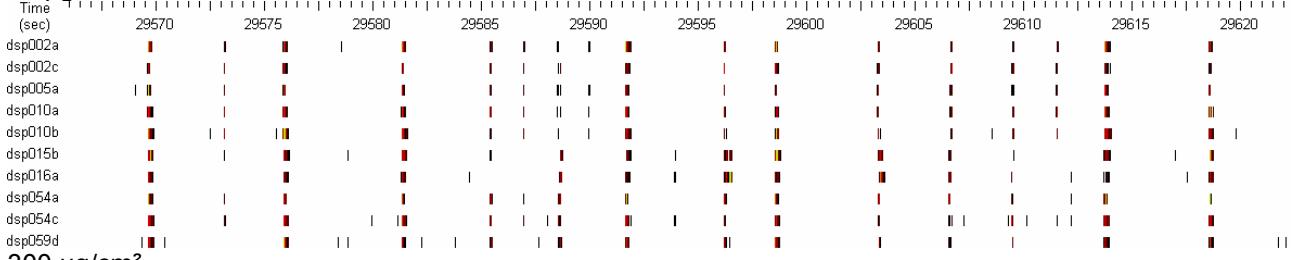


**Figure 4 (continued):** Concentration-response curves after cumulative application of carbon black nanoparticles.

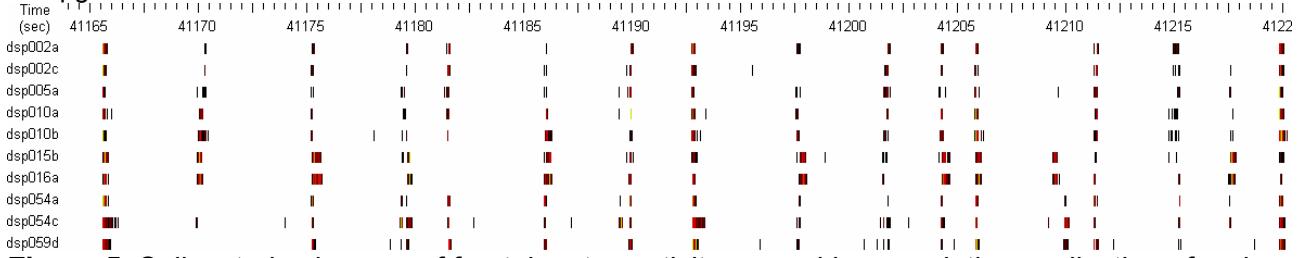
## Carbon black – native



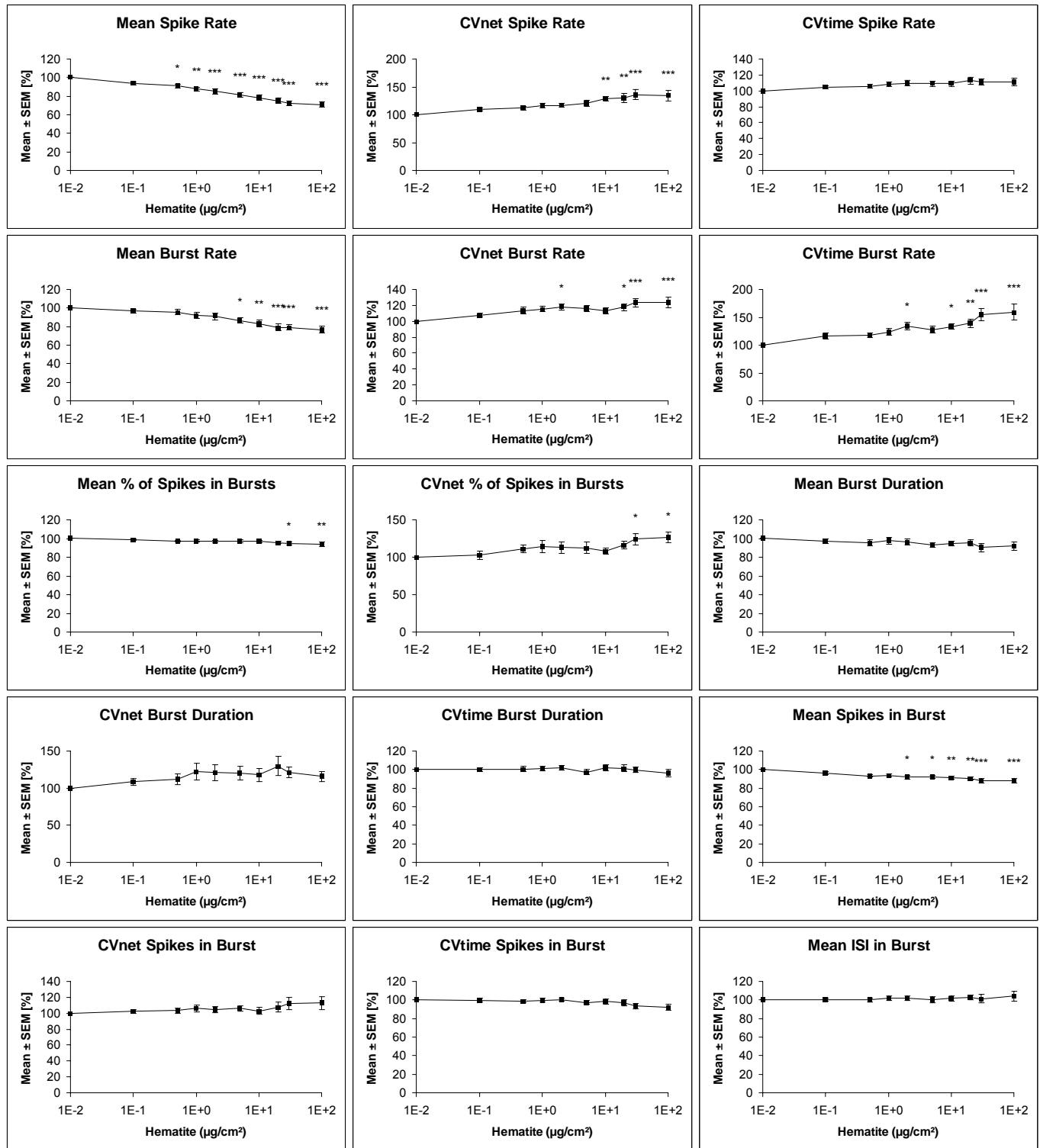
10 µg/cm<sup>2</sup>



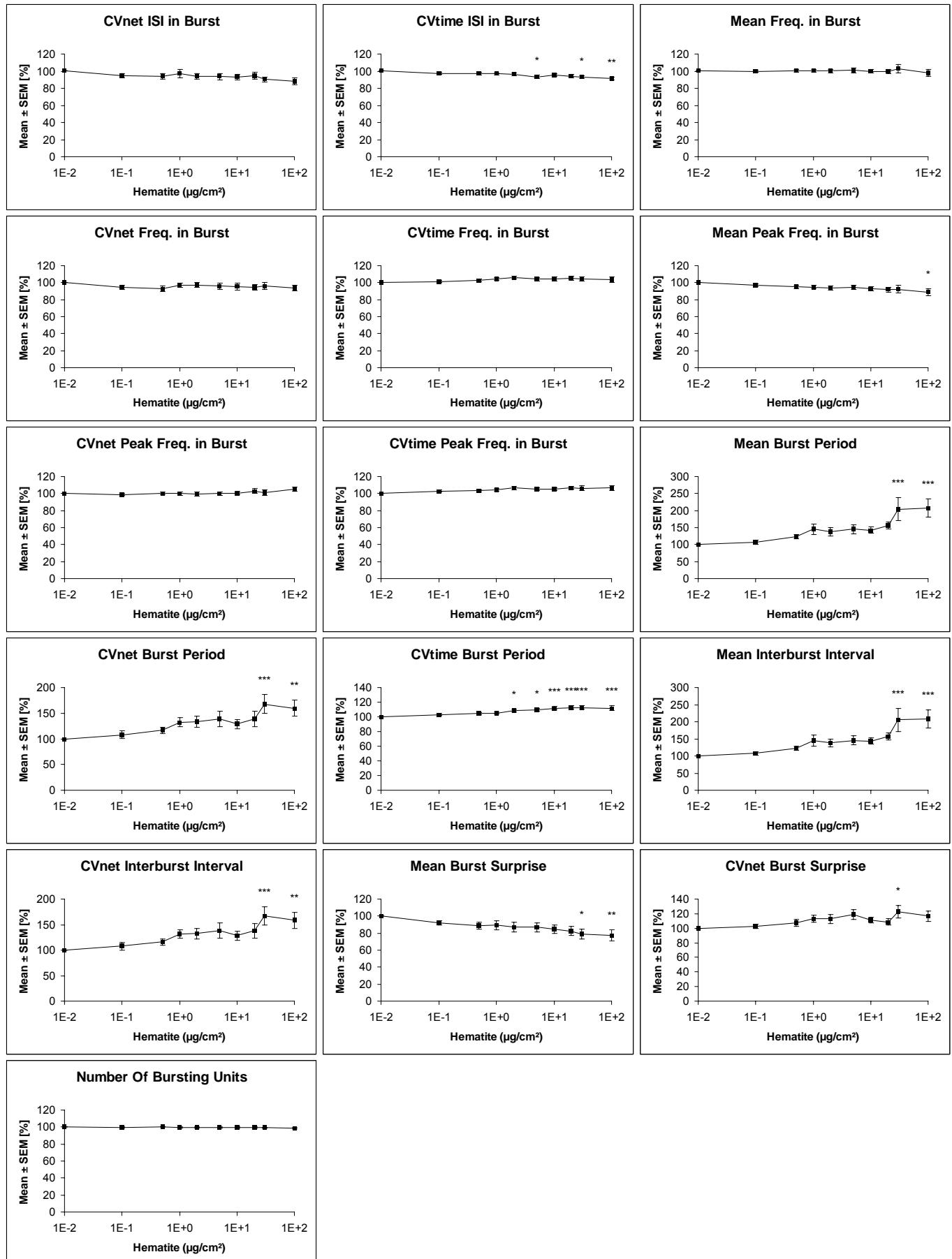
300 µg/cm<sup>2</sup>



**Figure 5:** Spikes train changes of frontal cortex activity caused by cumulative application of carbon black nanoparticles. Plotted are 60 s of 10 neurons from one network for native, one concentration representative for the *first phase* and one for the *second phase* activity.

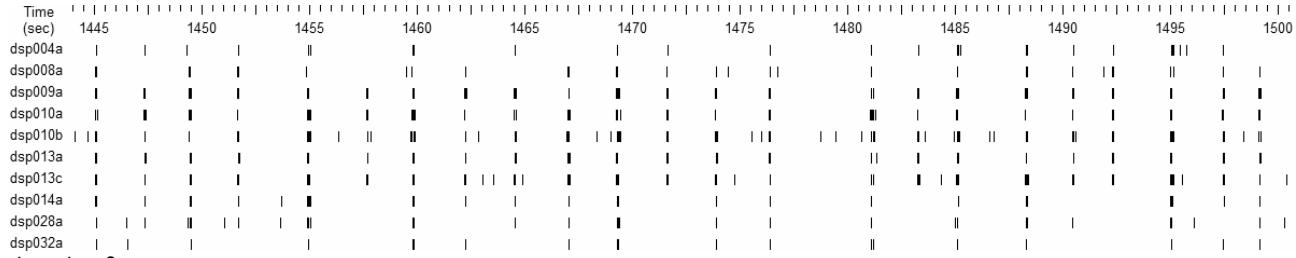


**Figure 6:** Concentration-response curves of 31 activity describing parameters after cumulative application of hematite nanoparticles. Significance levels after ANOVA Dunnett's test: \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

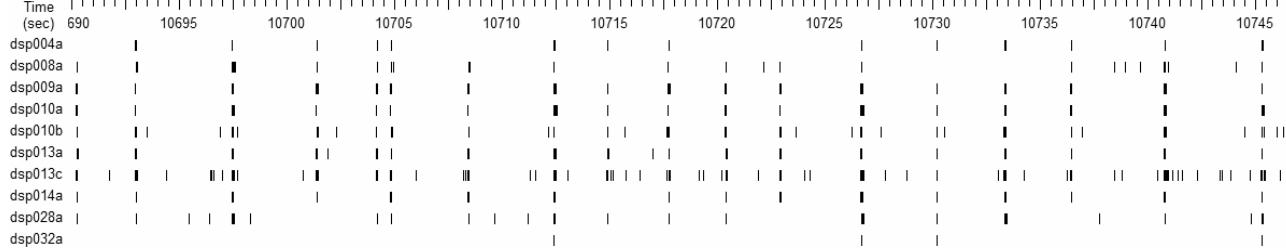


**Figure 6 (continued):** Concentration-response curves after cumulative application of hematite nanoparticles.

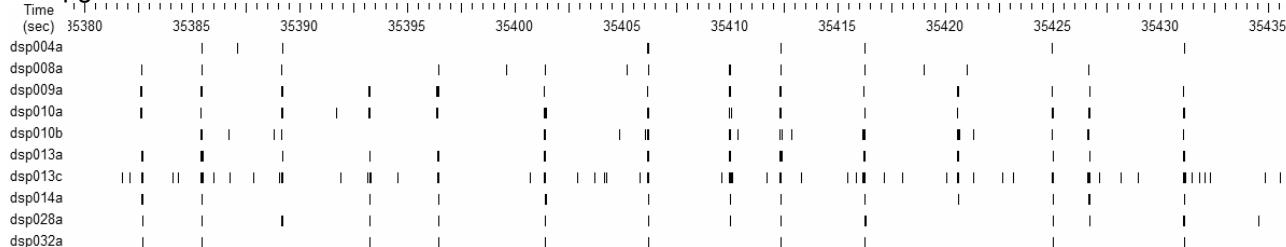
## Hematite – native



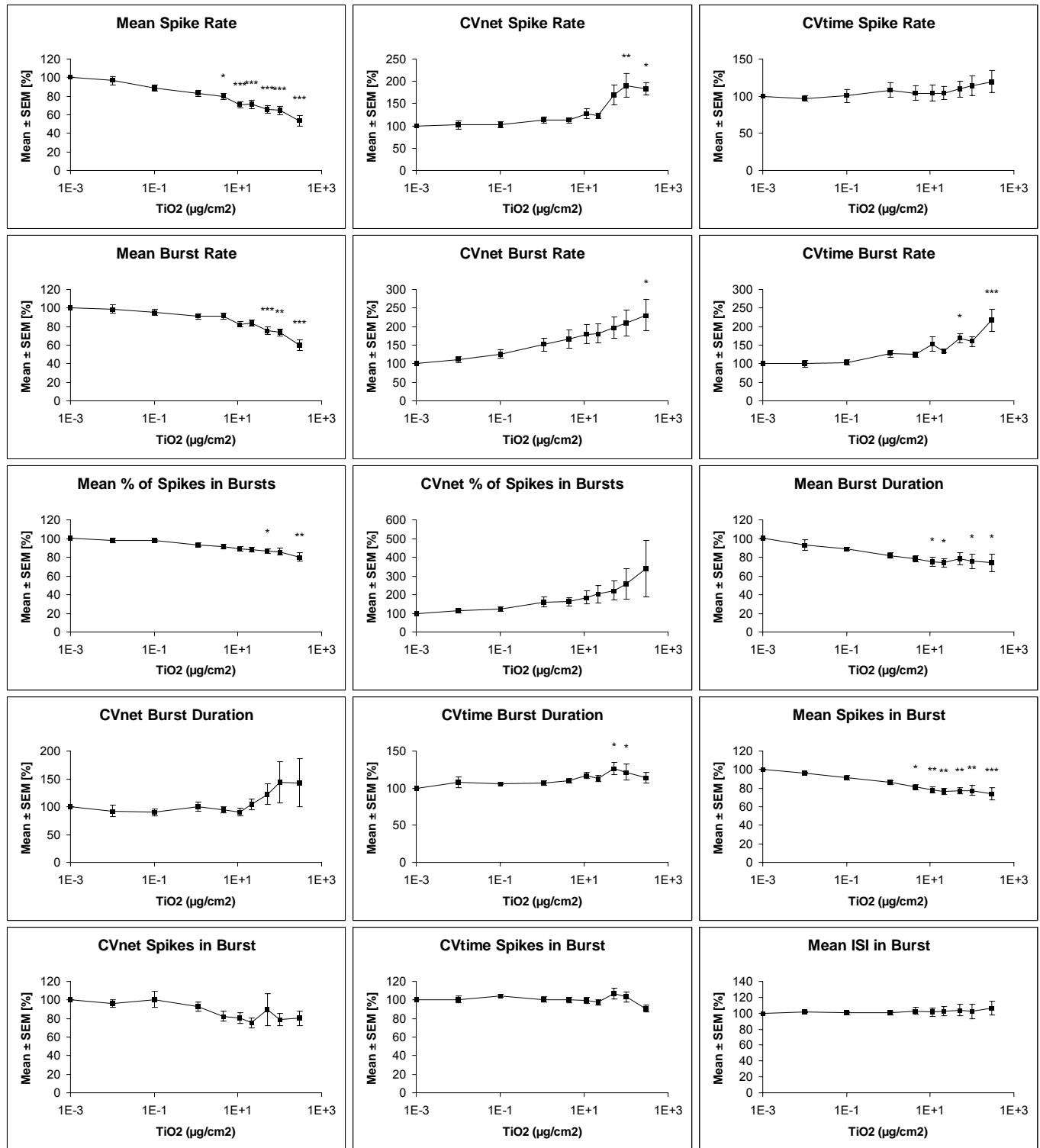
$1 \mu\text{g}/\text{cm}^2$



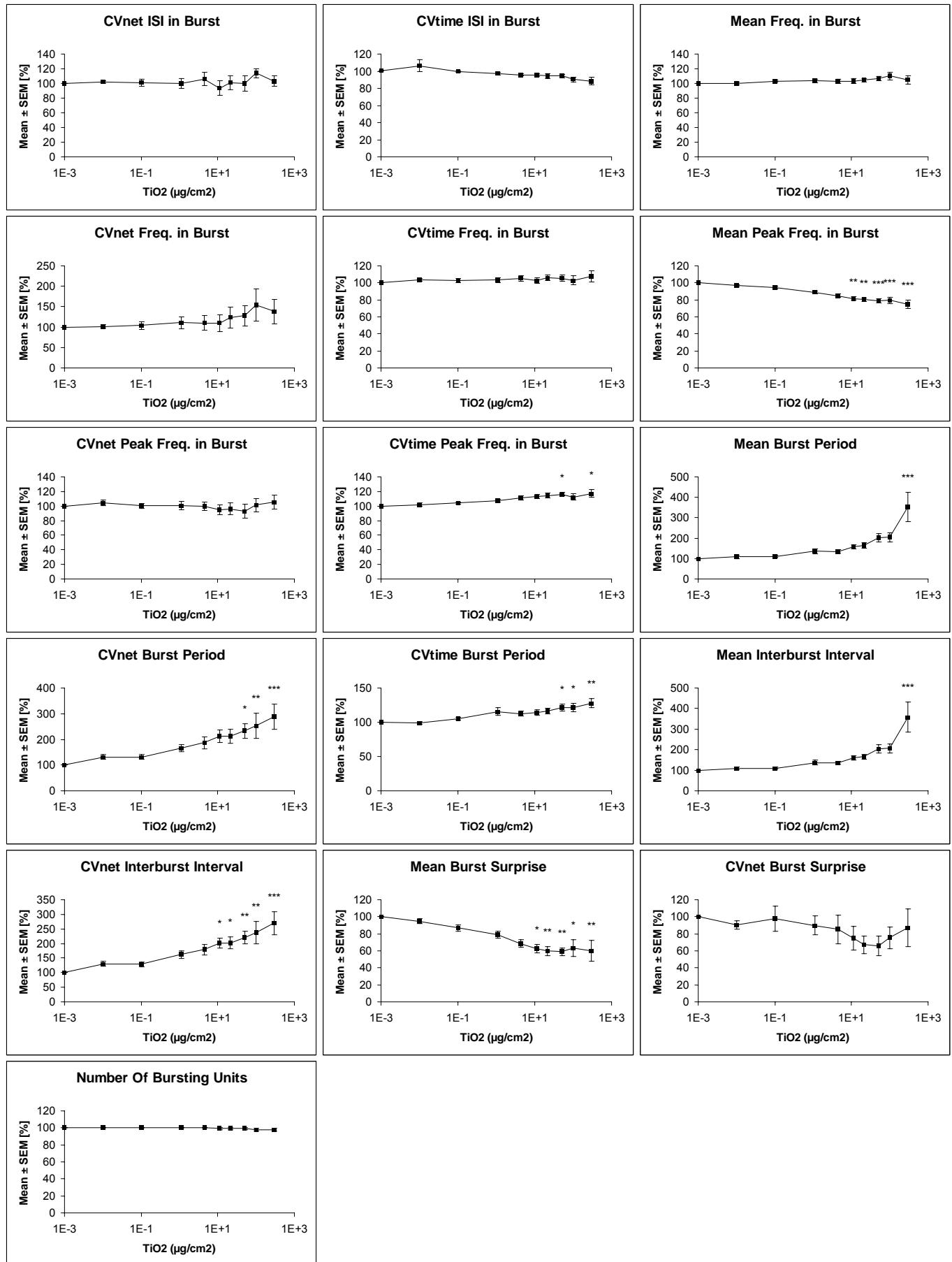
$300 \mu\text{g}/\text{cm}^2$



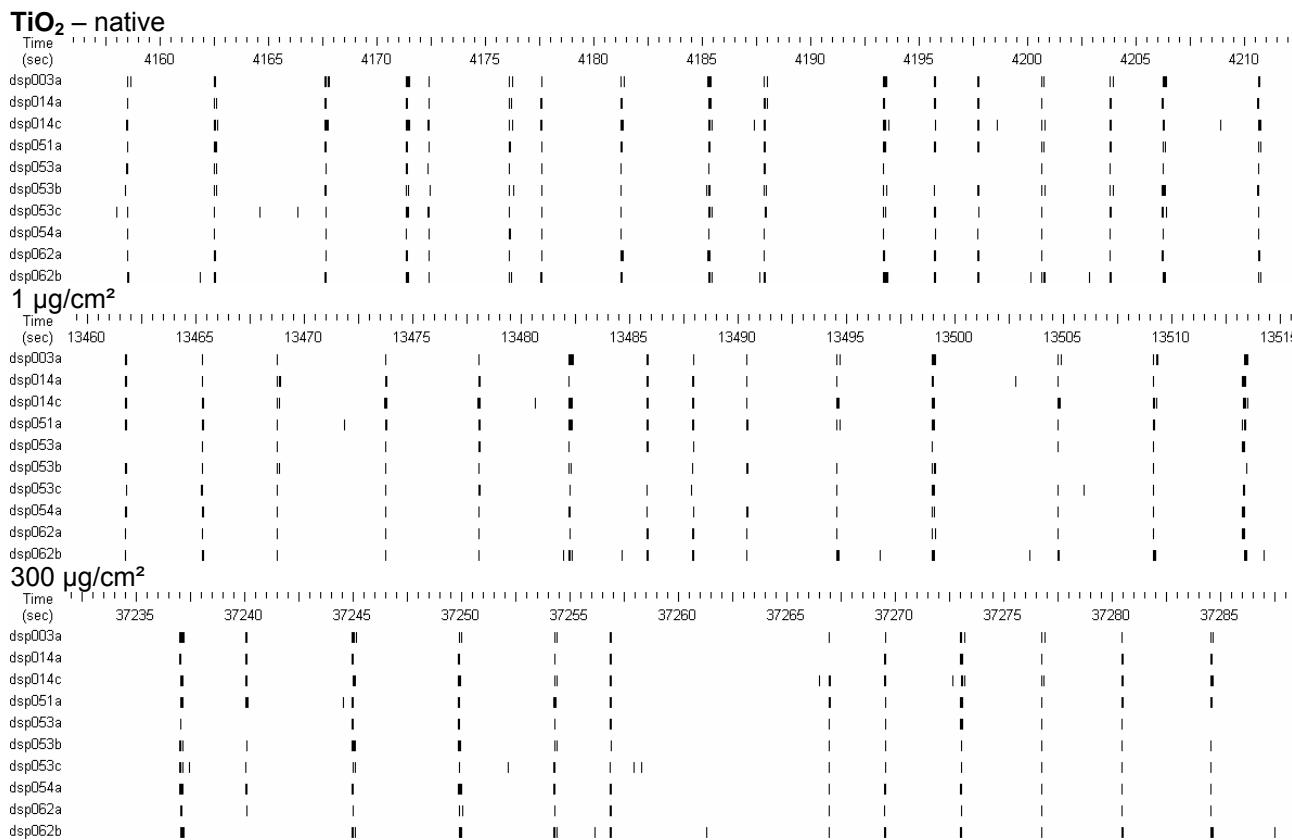
**Figure 7:** Spikes train changes of a frontal cortex activity caused by cumulative application of hematite. Plotted are 60 s of 10 neurons from one network for native and the concentrations related to the EC<sub>50</sub>, and EC<sub>90</sub> activity.



**Figure 8:** Concentration-response curves of 31 activity describing parameters after cumulative application of titanium dioxide nanoparticles. Significance levels after ANOVA Dunnett's test: \* p<0.05; \*\* p<0.01; \*\*\* p<0.001.



**Figure 8 (continued):** Concentration-response curves after cumulative application of titanium dioxide nanoparticles.



**Figure 9:** Spikes train changes of frontal cortex activity caused by cumulative application of titanium dioxide nanoparticles. Plotted are 60 s of 10 neurons from one network for native and the concentrations related to the EC<sub>50</sub>, and EC<sub>90</sub> activity.

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